Assessment of antioxidant, Cytotoxic and Apoptotic Profile of Potential Fraction (s) from Some Plants

A Synopsis

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1. INTRODUCTION:

Human body is composed of many millions of tiny cells, each a self-contained living unit. Normally, each cell coordinates with the others that compose tissues and organs of a body. Normal cells in the body grow and divide for a period of time and then stop growing and dividing. Thereafter, they only reproduce themselves as necessary to replace defective or dying cells. Cancer occurs when this cellular reproduction process goes out of control. In other words, cancer is a disease characterized by uncontrolled, uncoordinated and undesirable cell division (Evert, 2010). It is an abnormal growth of cells caused by multiple changes in gene expression leading to deregulated balance of cell proliferation and cell death and ultimately evolving into a population of cells that can invade tissues and metastasize to distant sites, causing significant morbidity and, if untreated, death of the host (Ruddon, 2007). Normal cells become cancerous through a process called *transformation*, leading to the uncontrolled growth of the cancer cells, which produces a *tumour* or *neoplasm* (Panno, 2005). Tumour or *neoplasm* is a relentlessly growing mass of abnormal cells. As long as the neoplastic cells remain clustered together in a single mass, the tumour is said to be benign, and a complete cure can usually be achieved by removing the mass surgically. A tumour is counted as a cancer only if it is malignant, that is, only if its cells have the ability to invade surrounding tissue (Alberts B, 2002). Cancers are classified according to the tissue and cell type from which they originate. Cancers arising from epithelial cells are termed carcinomas; those arising from connective tissue or muscle cells are termed sarcomas and those derived from haemopoetic cells are termed leukaemia (Alberts B, 2002). About 90 % of all cancers are carcinoma (Hopkins, 2010).

Cancer is a clonal disease arising by the multistep accumulation of genetic mutations in tumour-suppressor genes; oncogenes and DNA repair genes that favour expansion of the new clone over the old – a process akin to Darwinian evolution. Natural selection will favour expansion of a given clone that carries advantageous characteristics. These changes allow a normal cell to achieve the "hallmark" features of cancer, which have been summarized as (Hanahan and Weinberg, 2000);

a. Capacity to proliferate irrespective of exogenous mitogens;

b. Refractoriness to growth-inhibitory signals;

c. Resistance to apoptosis s;

d. Unrestricted proliferative potential (immortality);
e. Capacity to recruit a vasculature (angiogenesis);
f. Ability to invade surrounding tissue and eventually metastasize.

Apoptosis, or programmed cell death, is a normal component of the development and health of multicellular organisms. Cells die in response to a variety of stimuli and during apoptosis they do so in a controlled, regulated fashion. This makes apoptosis distinct from another form of cell death called necrosis in which uncontrolled cell death leads to lysis of cells, inflammatory responses and, potentially, to serious health problems. Defective apoptosis (programmed cell death) represents a major causative factor in the development and progression of cancer. Hence, targeting apoptosis for the treatment of cancer is major area of interest.

According to American cancer society, about 1,596,670 new cancer cases are expected to be diagnosed and about 571,950 Americans are expected to die of cancer (more than 1,500 people a day) in 2011. Cancer is the second most common cause of death in the US, exceeded only by heart disease. In the US, cancer accounts for nearly 1 of every 4 deaths (cancer facts & figures, 2011). The latest report prepared by Indian council of medical research revealed that, there are about 25, 00,000 cancer patients in India and every year there is an increment of 10,000 new cancer patients. The risk for developing cancer increases with age. More than three-fourth (77 percent) of all cancers are diagnosed in people 55 years of age or older. According to WHO around 10 million new patients diagnosed with cancer every year.

There are various modalities of cancer treatment which mainly includes surgery, chemotherapy and radiation therapy. Chemotherapy is the use of drugs to eliminate rapidly proliferating cancer cells. One of the extremely promising strategies for cancer management is chemoprevention, which is defined as the use of natural agents (alone or in combination) obtained from medicinal plants to prevent the development of cancer in humans. Multidisciplinary scientific investigations are making best efforts to combat this disease, however a convenient cure remain elusive.

It has been estimated that about 50% of the prescription products in Europe and USA are originating from natural products or their derivatives (Newman et al., 2003). Out of the 250,000–500,000 plant species on earth, only 1–10% have been studied chemically and pharmacologically for their potential medicinal value (Verpooete, 2000). In spite of the recent domination of the synthetic chemistry as a method to discover and produce drugs, the potential of bioactive plants or their extracts to provide new and novel products for disease treatment and prevention is still enormous (Kviecinski et al., 2008).

Anticancer research has the greatest impact of plant derived drugs, where drugs like vinblastine, vincristine, taxol, and camptothecin (Wall, 1980)mhave improved the chemotherapy of some cancers (Newman et al., 2003). Plants have an almost unlimited capacity to produce substances that attract researchers in the quest for new and novel chemotherapeutics (Reed and Pellecchia, 2005). The continuing search for new anticancer compounds in plant medicines and traditional foods is a realistic and promising strategy for its prevention (YW, 2009). Numerous groups with anticancer properties are plant derived natural products including alkaloids, phenylpropanoids, and terpenoids (Park et al., 2008).

India represented by rich flora of medicinal plants, tradition and natural biodiversity, offers a unique opportunity for drug discovery researchers (Gautam et al., 2007). This knowledge-based country is well recognised for its heritage of the world's most ancient traditional system of medicine, Ayurveda (Gautam et al., 2009). We, in India have 2 (Eastern Himalayan and Western Ghat) of 18 hotspot of plant biodiversity in the world. Interestingly, we are seventh among the 16 mega diverse countries, where 70% of world's species occur collectively (Gautam et al., 2007). Presently there is growing interest in pharmacological evaluation of various plants used in Indian traditional system of medicine. One of such plant is *cleome viscosa* Linn. belongs to family *Capparidaceae*. It is a weed distributed throughout the tropics of the world and the plains of India (Nadkarni, 1982). The plant was selected depending on the information gathered from traditional healers, reputed botanist and various available ethnobotanical literatures.

Free radicals are natural by-products of the body turning food into energy (ATP). The body has mechanism to deal with these free radicals, because they are dangerous and may attack the body

itself, damage key cellular molecule such as DNA (Florence et al., 1991). Cells with damaged DNA may be more prone to cancer (Berwick and Vineis, 2000). Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals (Dormandy, 1978). Free radical damage may lead to cancer (Ames, 1989). Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause. Considerable laboratory evidence from chemical, cell culture and animal studies indicates that antioxidants may slow or possibly prevent the development of cancer (Laura Dungo, 2011). Recent studies suggest that plant derived compound have a remarkable property to inhibit cancer cells proliferation *in vitro*. Similarly, many of these plant derived agents have the ability to induce antioxidant activity via generation of Reactive Oxygen Species and free radical scavenging activity. Production of ROS correlates with the induction of apoptosis in the cancer cells.

Cancer is a generic term that has been used to describe diseases in which cells behave abnormally and divide uncontrollably with potential of invading various tissues. It is one of the most dreaded diseases of the 20th century and spreading further with continuance and increasing incidence in 21st century (Balachandran *et al*, 2005). The relative roles of genetic make-up and environmental exposure in the causation of cancer have been a matter of debate (Toh *et al*, 2007). Geographic differences and trends over time in the risk of cancer implicate environmental exposures as major causal factors and often identify the responsible carcinogens (e.g., Tobacco, alcohol, radiation, occupational toxins, infections, diet, drugs) (Huang *et al*, 2007).

The disease affects various organs of the body and is a major cause of mortality worldwide. World Health Organization reported global mortality figures of 7.4 million for which cancers of lung, stomach, liver, colon and breast were the major contributors. Studies done in developed countries like United States have shown deaths attributable to cancer to be on decline since the 1990s (Edward *et al*, 2010). The global mortality rates though are still expected to climb up to 12 million by the end of 2030, 30% of which would have been preventable.

1.2 Plants profile:

1.2.1 Bombax malabaricum DC

Scientific classification

Kingdom : Plantae

Order : Malvales

Suborder: Gentianineae

Family : Bombacaceae

Subfamily: Malvaceae

Genus : *Bombax*

Species : *ceiba*

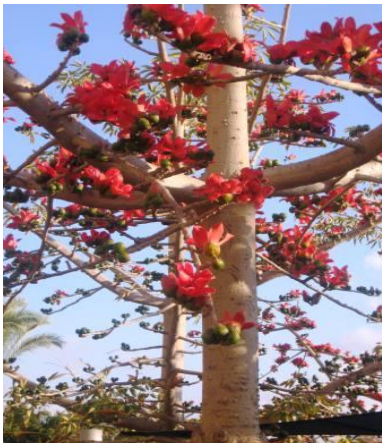

Fig.:- *Bombax malabaricum* DC

Picture capture from Rajasthan

Synonyms : *Bombax malabaricum* D.C., *Salmalia malabarica* Schott and Endl.

Habit and Habitat

Bombax malbaricum DC syn. *Salmalia Malabarica* Schott. And Endl., *B.ceiba* Linn. belongs to the family Bombacaceae. It is commonly known as Salmali, Moca, Picchila, Raktapushp, katakdhya, Tulini, semul, shimul, Shemalo, Semal, kapok, Silk cotton tree. It is deciduous tree attaining a height upto 40 meters and a girth up to 6 meters or more and In India; it is distributed throughout the hotter parts of the country upto 1500 meter or more (The Ayurvedic Pharmacopoeia of India). Its young stems and branches are covered with stout and hard pickles; its leaves are large, spreading, glabrous and digitate; leaf lets are 5-7, lanceolate and 10-20 cm long; its flowers are numerous, large, fleshy, bright crimson, yellow or orange containing many seeds with long, dense silky hairs (Compendium of Indian medicinal plants).

1.2.2 *Cleome viscosa* Linn.

Scientific classification

Kingdom : Plantae
Division : Angiosperms
Sub Division : Dicotyledonae
Class : Polypetalae
Series : Thalamiflorae
Order: Parietales
Family: Capparidaceae
Genera: *Cleome*
Species : *viscosa*
Botanical name : *Cleome viscosa Linn.*

Fig.: *Cleome viscosa* Linn.
Picture capture from Rajasthan

Habit and Habitat

Cleome viscosa Linn. (Capparidaceae) is commonly known as Tickweed, wild mustard or Spider plant (English), Hurhur (Hindi) and Hurhuria (Bengali). It occurs in woodland and grassland, and is a weed of fallow land, fields, roadsides and wasteland, often occurring on sandy soils, but sometimes on calcareous and rocky soils. It is a widely distributed herb with yellow flowers and long slender pods containing seeds. The whole plant is sticky in nature and has a strong odour resembling asafoetida. It is found in both seasonal dry and humid conditions, from mean sea level up to an altitude of 1000 m. The seeds have no dormancy and germinate readily after shedding. Plants start flowering 3–4 weeks after germination and the lifecycle is about 3 months. Cleome viscosa Linn (Capparidaceae), also called “Dog mustard”, is a herb that grow up to 1m height in India (Parimala et al., 2004). Cleome is a large genus included in the Capparaceae family, which comprises 427 species occurring in tropical and subtropical regions of the world (Brummit, 1992). This is the herb having yellow flowers and pods which are long and cylindrical and contains seeds. The color of seeds of the plant Cleome viscosa is brown to slightly black (Anburaj jeyaraj et al., 2011)

1.2.3 *Wrightia tinctoria Roxb.*

Scientific classification:-

Kingdom : Plantae

Order : Gentianales

Family : Apocynaceae

Genus : Wrightia

Species : *Wrightia tinctoria*

Botanical name : *Wrightia tinctoria*

Fig.:- *Wrightia tinctoria*

Picture capture from Rajasthan

Vernacular names:

Hindi: Indarjau, Sanskrit: Kutajah, English : Ivory wood, pala indigo plant.

Habit and Habitat

Wrightia tinctoria R.Br. (Gentianales, Apocynaceae) (*Wt*) is a medium-sized evergreen tree producing milky-white latex. The botanical description of the 23 species of the genus *Wrightia* was done by Ngan (1965) (Ngan PT, 1965) which also includes *Wrightia tinctoria* and its subspecies. Leaves are opposite, petiolate, glands axillary and glabrous. Bark is smooth and ivory colored. Inflorescence is terminal and flowers are white, bisexual, actinomorphic and hypogynous.(Raju AJS et al.,2005) Seeds have hairs at the chalazal end.(Brown R, 1995) Reproduction is autogamous. Pollination is entemophilous without any direct contact of the biotic factor with pollen or stigma. (Raju AJS et al.,2005, Reddi CS et al., 1979) Fruits are mericarpous in origin, dark green in color and have paired pendulous follicles joined at the tips which may be long upto 50 cm. The plant produces white latex. *Wt* is generally propagated by seeds and stem cuttings. (Rao PS et al.,1999, Suresh DR et al., 2004) The wood from *Wt* has uniformly distributed grains and is light in weight. It grows in wide range of soil types and are often found to grow as undergrowth in forest and under various plantations like oil plantation etc.(Sarada S et al., 2002) The karyotype of *Wt* (intraspecific polyploidy, disomy) is 2n=20, 22.[Gill BS et al.,1990, Khan K et al. 2011) They are found in India, Myanmar, Australia, tropical Africa, Nepal and China.(Orwa C et al., 2009)

2. AIM AND OBJECTIVES

In spite of advance made in present day medicinal science, the general achievement rate has stayed unassuming and inadmissible. Reduction of restricted term and return of manifestations are visit because of an assortment of inadequacies related with exhibit day drugs. Ordinary inadequacies related with present day allopathic treatment incorporate unnecessary systemic poisonous quality, Lack of cell specificity, because of the absence of the cell specificity tranquilize application will be too much inefficient, and solid tissues will be presented to lethal symptoms. As a result of these inconveniences related with the advanced treatment there is a more prominent intrigue is expanding towards natural medications acquired from different plant sources. The current investigate worth of effort incorporates identification, extraction, isolation; characterization and pharmacological assessment from claiming anticancer Furthermore antioxident possibility from claiming of *Bombax Malabaricum* DC, *Cleome Viscosa* Linn. Also, *Wrightia Tinctoria* Roxb are planned to search for protected and effective anticancer and antioxidant.

1. To collect Plant Material for identification and authentification.

2. To perform successive extraction of plant material.

3. To perform preliminary phytochemical screening of the extracts for the detection of various phytoconstituents (Alkaloids, steroids, tannins, flavonoids, saponins, carbohydrates, fatty acids etc).

4. To perform the pharmacological activity (in-vitro antioxidant and anticancer) of crude drug extracts by employing on wistar albino rats using different aphrodisiac rat models.

5. To perform the acute toxicity study (according to OECD test guidelines 425) of crude drug extracts by employing on wistar albino rats.

6. To perform the pharmacological activity (*ex-viva* antioxidant and *in-viva* anticancer) of crude drug extracts by employing on wistar albino rats using different antioxidant and anticancer rat models.

3. REVIEW OF LITERATURE

- **Boddapati Srinivasa Rao et al. (2014)** reported that **t**he neuroprotective effect of ethanolic extract of *Cleome viscosa* (EECV) was investigated against streptozotocin (STZ) induced diabetic neuropathy in Wistar rats. Intraperitoneal injection of STZ resulted in significant increase in thermal hyperalgesia and hyperlipidemia after four weeks. Antioxidant enzyme [superoxide dismutase (SOD), glutathione (GSH) and catalase] levels were reduced and malondialdehyde (MDA) level was increased significantly in diabetic rats as compared to the vehicle control rats. Four weeks of chronic treatment with Cleome viscosa extract attenuated the level of nociceptive threshold significantly and dose dependently. It also decreased the elevated levels of lipids, lipid peroxidation, and oxidative stress significantly and dose dependently. Many studies provide investigational evidence of the protective effect of EECV on nociception, hyperlipidemia and oxidative stress in STZ induced diabetic neuropathy.

- **Upadhyay aadesh et al. (2014))** evaluated that *Cleome viscosa* Increases the Expression of Basic Fibroblast Growth Factor and Type III Collagen in Rat Cutaneous Wound after topical application- The present study explored the wound healing property of methanolic extract of C. viscosa using Wistar rat cutaneous excision wound model. The rate of wound contraction, quantification of hydroxyproline and histopathological examination of wound granulation tissue was performed to assess the wound healing property. From this study it was revealed that the topical application of methanolic extract of *Cleome viscosa* (2.5% w/w) significantly accelerated the wound contraction rate (95.14%, 24 postoperative days), increased the hydroxyproline content (3.947mg/100mg tissue), and improved histopathology of wound tissue as compared to control groups.

- **Sangeetha S et al. (2014), Aparadh V.T. et al (2012) and Khadiza khanam et al. (2015)** evaluated on the whole plant cleome viscosa showed that the plant contains some bioactive principles that possess strong antioxidant activity along with strong anti-inflammatory activity, analgesic activity, and moderate CNS depressant activity. Therefore, the plant of *Cleome viscosa* could be considered as an important source of antioxidant activity.

- **Faizan mohammad and Mazumder avijit (2013)** reported that the leaves and whole plant of Cleome viscosa are used as a folk remedy to cure the wounds, ulcers, inflammations and skin infections. The present investigation was undertaken to evaluate the wound healing property of the leaves and whole plant of *Cleome viscosa* on the experimentally induced excision wound model in rats. The studies on the wound healing models revealed that the methanolic extract of *Cleome viscosa* possesses significant wound healing activity.

- **Kumar DL et al. (2011)** have reported alpha glucosidase inhibitory activity of W. tinctoria. Results showed that the bark ethanol extracts have significant in vitro intestinal alpha glucosidase inhibitory activity at 1500 mcg/mL.

- **Dama GY et al. (2011)** reported that minimum time taken by the methanol extract of W. tinctoria leaves (100 mg/mL) were 13.97 and 23.3 min to cause paralysis and death of the worms respectively.

- **Aleykutty NA et al. (2011)** studied analgesic and anti-inflammatory effects of W. tinctoria leaf extract in rats and mice. The anti-inflammatory effect was investigated by human red blood cell membrane stabilization method and carrageenan induced hind paw edema method. Ethyl acetate fraction showed 67.21% protection at concentration 400 mg/kg. The ethyl acetate fraction was studied for its analgesic effect on acetic acid-induced writhing test and hot plate method in mice and was found to be effective. Above findings justify the traditional use of W. tinctoria leaves against inflammation and pain in psoriasis, hemorrhoids and swelling gums.

- **Divakar MC and Lakshmi SD (2011)** evaluated antiulcer activity of leaves of W. tinctoria evaluated against experimentally induced acute gastric ulcer model in albino rat. The antiulcer activity of methanol was evaluated by comparing with carboxy methyl cellulose, pylorus control, aspirin and standard famotidine, by employing aspirin plus pylorus ligation induced ulcer model. The biochemical parameters such as volume of gastric juice secretion, pH, free acidity, total acidity, ulcer index and percentage inhibition were studied at the concentration of 200 mg/kg body weight. The plant methanolic extract showed significant gastro protective activity (65.89%), when compared with the standard drug famotidine (20 mg/kg). The results suggested that the methanolic extract of W. tinctoria leaves possess anti-ulcer effect.

- **Sathianarayanan et al. (2011)** investigated diuretic activity on male albino rats using water and alcoholic extract of leaves of W. tinctoria. The extracts showed a potent diuretic effect with increase in electrolyte concentration in urine, when compared to standard drug (frusemide) in albino rats.

- **Kumar et al. (2011)** evaluated the reducing power and free radical scavenging activity of ethanol extract of W. tinctoria bark. In vitro antioxidant evaluation was done by measuring the reducing power and inhibition of superoxide production. The study demonstrates that the ethanol bark extract has significant antioxidant and free radical scavenging activity.

- **Raman R Chandak et al. (2010)** reported that alcohol and aqueous extracts from the leaves of *Cleome viscosa* showed anthelmintic activity against Pheretima Posthuma and Ascardia galli. Three concentrations (50, 100 &150mg/ml) of each extract were studied in activity, which involved the determination of the time of paralysis and time of the death of the worm. Both the extracts exhibited significant anthelmintic activity at the highest concentration of 150 mg/ml. Albendazole in the same concentration as that of extract was included as the standard reference and distilled water as a control. The anthelmintic activity of alcohol and aqueous extracts of *Cleome viscosa* has therefore been demonstrated for the first time.

- **V. Jain et al. (2011)** reported that methanolic extract of roots of B. malabaricum was evaluated using DPPH (1, 1-diphenyl-2-picryl- hydrazyl) radical scavenging assay and reducing power assay. Methanolic extract of the roots showed a very good DPPH radical scavenging activity in a dose dependant manner. Research concludes that antioxidant activity was due the higher percentage of phenolic compounds and tannins. The reducing ability of methanolic extract was measured by transformation of Fe3+ to Fe2+ in the presence of extract at 700nm. The extract showed dose dependant reduction ability in reducing power assay.

- **Hossain E et al. (2011)** reported that the antipyretic activity of methanolic extract of *Bombax malabaricum* leaves. The methanolic extract showed significant antipyretic activity in the doses of 200 mg/kg (p\0.05) and 400 mg/kg (p\0.01) in Baker's yeast-induced pyrexia and was higher

than control. The maximum antipyretic activity for the extract occurred at 6 h while that of paracetamol, the reference standard, occurred at 3 h.

- **Raj RA et al. (2010)** investigated and reported the antidiabetic effect of petroleum ether extract of W. tinctoria leaves in streptozotocin induced diabetic rats.

- **Ponnusamy K et al. (2010)** investigated Indirubin isolated from the chloroform extract of leaves of W. tinctoria tested for its antibacterial activity on S. aureus, P. aeruginosa etc. and efflux pump inhibitory (EPI) activity. Indirubin showed antibacterial activity against both Gram-positive and Gram-negative strains; and drug resistant to S. aureus; the observed MIC was 12.5 mg/mL for S. aureus and 25 mg/mL for Streptococcus epidermidis. W. tinctoria had significant EPI property and further clearly showed the synergistic effect along with ciprofloxacin. This indicated that indirubin has the capacity to block the Nor A efflux pump, which leads to cell susceptibility, due to increasing concentration of ciprofloxacin within the cell increasing concentration of ciprofloxacin within the cell. Pityrosporum ovale (P. ovale) is a yeast causing infection of skin and scalp leading to dandruff.

- **Jayechandran R et al. (2010)** screened the antibacterial potential of W. tinctoria using various solvents viz., hexane, petroleum ether, ethyl acetate, chloroform, acetone and methanol on bacterial cultures of E. coli (MTCC 1195), Klebsiella pneumoniae (MTCC 2405), Enterobacter aerogens (MTCC 2823), S. typhi (MTCC 733), Proteus vulgaris (MTCC 1771), P. aeruginosa (MTCC 2642), S. aureus (MTCC 1430), Bacillus cereus (MTCC 1272). Petroleum ether, chloroform, acetone and hexane extracts showed the highest inhibition zone between 19-22 mm against E. coli, Proteus mirabilis and Klebsiella pneumoniae, respectively, while the moderate antibacterial activity was attributed to hexane, acetone and chloroform extracts.

- **Shankara SR et al. (2010)** studied , hexane, methanol, chloroform, ethyl acetate and water extracts of leaves of W. tinctoria were subjected to in vitro antibacterial screening against plant pathogenic bacteria using disc diffusion method. Results showed that ethyl acetate and methanol extracts have significant antibacterial activity against Gram-negative bacteria with MIC 50 µg/mL for Xanthomonas campestris and Erwina sp.

- **Shruthi A et al (2010)** studied anthelmintic activity of crude petroleum ether and chloroform extracts of leaves of W. tinctoria using Pheretima posthuma as a model organism were studied at three concentrations (2.5, 5.0, 7.5 mg/mL) of each extract. Petroleum ether and chloroform extract of W. tinctoria caused significant paralysis (125.83 and 94.5 seconds) and death (162.33 and 140.28 seconds) of Pheretima posthuma respectively.

- **Tharkar PR et al. (2010)** investigated for anti-inflammatory activity of bark in carrageenan-induced rat paw oedema and cotton pellet induced granuloma animal models. The results of this study exhibited that the aqueous and chloroform extract treated group showed significant ($P<0.05$) reduction in paw oedema from 4-5 h, while methanolic extract and diclofenac sodium treated group showed a significant reduction ($P<0.05$) in paw oedema from 2-5 h when compared to control group.

- **Jain PS and Bari SB (2010)** investigated anti-inflammatory activity of petroleum ether and methanol extracts of W. tinctoria bark using carrageenan and histamine-induced paw edema animal models. The results of this study exhibited that the extract possessed significant dose dependent anti-inflammatory activity.

- **Jain PS and Bari SB (2010)** reported the potential effect of petroleum ether and methanolic extracts of bark in wound healing in Wistar albino rats.

- **Raman R Chandak et al. (2010)** reported that alcohol and aqueous extracts from the leaves of *Cleome viscosa* showed anthelmintic activity against Pheretima Posthuma and Ascardia galli. Three concentrations (50, 100 &150mg/ml) of each extract were studied in activity, which involved the determination of the time of paralysis and time of the death of the worm. Both the extracts exhibited significant anthelmintic activity at the highest concentration of 150 mg/ml. Albendazole in the same concentration as that of extract was included as the standard reference and distilled water as a control. The anthelmintic activity of alcohol and aqueous extracts of *Cleome viscosa* has therefore been demonstrated for the first time.

- **Bigoniya P. Rana AC (2010)** investigated the hepatoprotective effect of triterpene fraction isolated from the stem bark of W. tinctoria (containing lupeol, β-amyrin and β-sitosterol) against

CCl_4-induced hepatotoxicity in comparison with known standard sylmarin in the rat. Pretreatment with triterpene fraction 125, 250 and 400 mg/kg, p.o. once a day for 4 d before CCl_4 and continued further 3 d, attenuated the CCl_4-induced acute increase in serum serum glutamic pyruvic transaminase, serum glutamic-oxaloacetic transaminase and alkaline phosphatase activities and considerably reduced the histopathological alterations. Further, triterpene fraction reduced thiopentone-induced sleeping time, suggesting the protection of liver metabolizing enzymes. The authors claim triterpenes administration changed the tissue redox system by scavenging the free radicals and by improving the antioxidant status of the liver replenished the depleted hepatic glutathione and superoxide dismutase. Triterpene pretreatment improves bromsulphalein clearance of the CCl_4-intoxicated liver and increases the cellular viability. These effects substantiate protection of cellular phospholipid from peroxidative damage induced by highly reactive toxic intermediate radicals formed during biotransformation of CCl_4. Triterpene fraction afforded protection against the hepatic abnormalities due to the presence of lupeol and β-amyrin. The study supports the traditional use of W. tinctoria bark in treatment of liver diseases.

- **Bhuvaneshwar Upadhyay et al. (2010)** reported an ethnomedicinal and ethnopharmaco-statistical studies in Eastern Rajasthan shows multiple uses of *B. malabaricum*. The tender twig was used as a toothbrush to cure mumps. Powdered flowers along with honey were used for menorrhagia. Root bark extract were used as a tonic in case of sexual debility and also as nervine rejuvenator. Root powder mixed with sucrose and milk was taken to avoid impotency.

- **Ravi V et al. (2010)** reported that the hepatoprotective activity of a methanolic extract of flowers of *B. malabaricum* was investigated. There was a signifcant decrease in alkaline phosphates (ALP), alanine transaminases (ALT), aspartate transaminases (AST) and total bilirubin levels, but increase in the level of total protein in comparison to control. MEBC signifcantly decreased the level of TBARS (thiobarbituric acid reactive substances) and elevated the level of GSH (reduced glutathione) at all doses as compared to control. The results obtained from the analysis of biochemical parameters and histopathological studies, resulted in the conclusion that the MEBC were not able to completely slip back the hepatic injury induced by INH and RIF, but it could limit the effect of INH and RIF to the extent of necrosis.

- **Raj RA et al. (2009)** investigated the hypoglycemic and hypolipidemic activity of petroleum ether extract of W. tinctoria in alloxan induced diabetes in albino Wistar rats. Oral administration of petroleum ether leaves extracts at doses 200 and 400 mg/kg exhibited a significant reduction in elevated levels of serum glucose, total cholesterol, and triglycerides. The results of this work confirmed W. tinctoria have significant application in diabetes mellitus illness and its related complications.

- **Khyade MS et al. (2009)** reported that aqueous extract with S. aureus (12 mm) and E. coli (14 mm). The antibacterial study of different solvent extracts such as petroleum ether, chloroform, acetone and methanol of W. tinctoria leaf has been carried out in our laboratory. It was a chloroform extract that exhibited the highest antibacterial activity against S. aureus (24 mm), E. coli, Salmonella typhi and Pseudomonas aeruginosa (P. aeruginosa), followed by acetone and methanol extracts. Interestingly, the observed antibacterial activity is almost equal, and/or more when compared to standard ampicillin.

- **Thabah P et al. (2009)** investigated the immunomodulatory activity of the bark extracts such as petroleum ether, ethanol and aqueous alcohol of W. tinctoria by using delayed type hypersensitivity reaction and carbon clearance assay. Petroleum ether and aqueous alcohol extracts (200 and 400 mg/kg, p.o.) produced a significant increase in delayed type hypersensitivity in response to sheep red blood cells. Petroleum ether extract showed better activity than aqueous alcohol in delayed type hypersensitivity response. Aqueous alcohol extracts at dose 200 and 400 mg/ kg, p.o., showed a significant dose dependent increase in the phagocytic activity. The results revealed that aqueous alcohol possesses immunostimulant activity in carbon clearance assays whereas the petroleum ether extract and aqueous alcohol showed immunomodulatory activity in the delayed type hypersensitivity model.

- **Bigoniya P, Rana AC (2009)** reported antidiarrheal potential of ethanol bark extract and isolated steroidal alkaloid fraction of W. tinctoria was investigated on different experimentally induced diarrhea models of rats, isolated rat ileum, and on enteric bacterium. The extract at 500 and 1 000 mg/kg dose, and steroidal alkaloid fraction at dose 50 and 100 mg/kg, significantly inhibited the frequency and wetness of faecal droppings in castor oil-induced diarrhea. Further,

extract and steroidal alkaloid fraction decreased propulsion of charcoal meal and reduced prostaglandin E2-induced enteropooling. Steroidal alkaloid fraction reduced amplitude, frequency, and tone of spontaneous gut movement; also inhibited acetylcholine-induced contraction of rat ileum. The results of this study confirmed good antidiarrheal activity of W. tinctoria against secretory, osmotic, motility related, and inflammatory diarrhea.

- **Selvam P et al. (2009)** have attempted antiviral activity and cytotoxicity of W. tinctoria. Different extracts of leaves selected against replication of HIV-1 (IIIB) in MT-4 cells and hepatitis C virus in Hue 5.2 cells. The cytotoxicity of leaves also tested against mock-infected MT-4 cells and Hue 5.2 cells. These extracts of the leaves have been evaluated for anti HIV-activity and cytotoxicity against HIV-1 (IIIB) replication in acutely infected MT-4 cells. None of the extracts exhibited anti-HIV activity in acutely infected MT-4 cells. However, the chloroform extract exhibited a maximum protection at 48% of the MT-4 cells against the cytopathic effect of HIV-1 (IIIB) at subtoxic concentration. The anti-hepatitis activity revealed that 50% effective concentration for the inhibition of hepatitis C virus subgenomic replicon replication in Huh 5.2 cells (luciferase assay) by chloroform extract was 10 μg/mL, whereas ether and ethanol extracts inhibits hepatitis C virus RNA synthesis at the concentration (EC_{50}) of 23 and 29 μg/mL, respectively. The concentration of extracts reduced the growth of exponentially proliferating Huh 5.2 cells by 50% was greater than 50 μg/mL.

- **S.Vimal Kumar et al. (2009)** reported that the extent of liver fibrosis was assessed by measuring the level of liver hydroxyproline, thiobarbituric acid, and serum enzymes levels. Following CCl_4 administration, hydroxyproline, thiobarbituric acid levels were significantly increased and total platelet was decreased and serum enzymes levels were elevated. Treatment with two different doses of the ethanolic extract of *Cleome viscosa* Linn reduces hydroxy proline, thiobarbituric acid and also the serum enzyme levels. The liver weight that increased following CCl_4 administration due to deposition of collagen was reduced by the ethanolic extract *Cleome viscosa* Linn. Thus, the results showed that the ethanolic extract of whole plant of Cleome viscosa Linn found to possesses Antifibrotic effect and that evidenced by the biochemical parameters.

- **Nima D et al. (2009)** ancient literature has revealed the traditional use of *B. malabaricum* as anti-inflammatory plants use by the Lohit community of Arunachal Pradesh. The paste of bark of *B. malabaricum* was used for anti inflammatory activities.

- **Ghimire K, Bastakoti RR (2009)** reported that bark juice of *B. malabaricum* was applied locally for the treatment of wounds; the bark juice was mixed with the bark juice of mango and guava and drunk to cure dysentery and intestinal spasm. The resins were also taken orally to treat worms and diarrhea; root juice was consumed to treat abdominal pain and gonorrhea.

- **M. A. H. Mollik et al. (2009)** an ethnobotanical survey of medicinal plants used by traditional practitioners and religious healers of Bangladesh has shown that seeds and roots of *B. malabaricum* were used in the treatment of serious skin diseases like Leprosy.

- **Vaghasiya Y and Chanda S (2009)** reported that strong antibacterial activity was shown by the methanol extracts of Salmalia malabarica. Methanol and acetone extracts showed potent antibacterial activity against *Klebsiella pneumonia*.

- **Chen Jun et al. (2009)** reported that pharmacological inhibitors of FAS activity preferentially repress cancer cell proliferation and induce cancer cell apoptosis without affecting nonmalignant fibroblasts. These made FAS an excellent drug target for cancer therapy. The cancer cell A549 was used as a cell model to test the inhibitory effort of flavonoid extracts on FAS.

- **Frederich M et al (2008)** was tested against Plasmodium falciparum. Further, tryptanthrin and several of its analogues were also tested for Plasmodium falciparum and showed very low IC_{50} values: 69 ng/mL for tryptanthrin and 0.43-10 ng/mL for analogues. These compounds were particularly active against atovaquone-, chloroquine- or mefloquine-resistant strains.

- **Bigonia P et al. (2008)** evaluated the ethanol (70%) extract of W. tinctoria bark showed significant analgesic activity in Eddy's hot plate reaction and tail flick method. The authors claim the observed analgesic activity could be due to the presence of steroids in the extract and concluded that W. tinctoria bark extract is effective against acute phasic pain.

- **Keshri G et al. (2008)** reported the bark extract plays an important role in infertility. The ethanol extract and its hexane-soluble, chloroform-soluble, water-soluble and water-insoluble fractions of the bark of W. tinctoria when administered orally during pre-implantation, periimplantation and early post implanation in adult female of Sprague-Dawley rats showed anti-implantation effect with moderate to potent estrogen-agonistic activity at 250 mg/kg dose; indicating antifertlity activity of extract. However, authors suggested that further studies on clinical and isolation of active natural products for development of newer agent from natural origin are required to identify contraceptive agents from natural sources lacking potent estrogenic activity.

- **Zito SW et al. (2007)** studied ocular aldose reductase inhibitory activity to W. tinctoria at 1 mg/mL as aldose reductase and sorbitol dehydrogenase to accelerate the formation of sorbitol from glucose in insulin-sensitive tissue leading to the development of cataracts in lens i.e. one of the common nephropathic condition in diabetes. The results of this study showed that W. tinctoria strongly inhibits aldose reductase (92.92%) with IC_{50} 5 μg/mL.

- **Bigonia P et al. (2007)** reported immunomodulatory activity of W. tinctoria bark alcohol extract on nonspecific and specific immune responses, studying the parameters such as survival study, carbon clearance test, delayed type hypersensitivity and hemagglutinating antibody titer. W. tinctoria extract at 400 mg/kg body weight increased the survival rate of rats against E. coli induced abdominal sepsis up to 15 d post infection. The extract showed significant homeopathic activity and raised neutrophils count significantly, which was further confirmed by increased phagocytic response against inert particles. W. tinctoria exhibited decreases in delayed type hypersensitivity response. Further, extract enhanced both primary and secondary humoral responses in rats sensitized with bovine serum albumin. The results of this study substantiate that W. tinctoria bark extracts have moderate non-specific immunostimulant.

- **Bawankule et al. (2007)** conducted an *in-vivo* investigation on coumarino-lignoids isolated from *Cleome viscosa* to evaluate its immunomodulatory potential. The results demonstrated that the coumarino- lignoids possessed significant immunomodulatory activity.

- **Mali et al. (2007)** have investigated the crude alcohol and aqueous extracts of the seeds of *Cleome viscosa* for their proclaimed anthelmintic activity using *Pheretima posthuma* and

Ascardia galli as test worms. Various concentrations (10–100 mg/mL) of each extract were tested in the bioassay, which involved the determination of time of paralysis and time of death of the worms. Both the extracts exhibited considerable anthelmintic activity in a dose-dependent manner. The most significant activity was observed at the highest concentration of 100 mg/mL against both types of worm.

- **Sengottuvelu S (2007)** a study was conducted to evaluate the hepatoprotective potential of the aqueous seed extract of *Cleome viscosa* against carbon tetrachloride (CCl_4)-induced liver damage in Wistar rats. The extract (200 mg/kg) was administered orally to the animals with hepatotoxicity induced by CCl_4, and silymarin (200 mg/kg) was given as the reference standard. The extract-treated group of rats showed a significant reduction in serum enzyme aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase, and lipid peroxidase and an increase in reduced glutathione (GSH) when compared to the CCl_4-treated control group. In addition, histopathological observations supported the significant protection of the extract, similar to that of the positive control, silymarin.

- **Kannan P et al. (2006)** screened different solvent extracts against skin bacteria and dermatophytes. The methanol and ethanol extracts were found to be more active against studied bacteria (at MIC 0.5 mg/mL for Bacillus subtilis, Staphylococcus epidermidis and 0.25 mg/mL for Staphylococcus aureus) whereas, hexane extract found to be potent against dermatophytic fungi such as Trichophyton rubrum and Trichophyton tonsurans at 2 mg/mL.

- **Dang R et al. (2006)**, reported that *in-vitro* antibacterial activities of the petroleum ether, chloroform, methanol and water extracts of leaves of W. tinctoria were studied against Staphylococcus aureus (S. aureus) (Gram-positive) Escherichia coli (E. coli) (Gram negative) as test organisms. The chloroform extract of W. tinctoria registered the highest zone (24 mm) against E. coli, followed by aqueous extract with S. aureus (12 mm) and E. coli (14 mm).

- **Krishnamoorthy JR et al. (2006)** studied *in-vitro* and *in-vivo* antifungal effect of the Dano, a poly-herbal hair oil, containing extracts of W. tinctoria, Cassia alata and Azadirachta indica. The results of this study also showed that Dano inhibited the growth of P. ovale and Candida albicans

with MIC at 30 mg/mL and 50 mg/mL respectively. Further clinical studies on 10 volunteers with severe dandruff in the age group of 18-22, showed mild traces to nil scaling with successive days of treatment.

- **Vijaykumar R et al. (2006)** carried out an investigation of W. tinctoria leaf extracts along with other species for the antimycotic activity against P. oval and showed the antifungal property as they progressively inhibited the growth of P. ovale on saboured dextrose agar medium.

- **Bawa AS et al (2006)** reported the leaves revealed different constituents such as flavonoids, steroids, glycosides, saponins, sterols, tannins and phenolics. Phenolics possess various biological properties such as antioxidant, antiinflammatory and estrogenic activities.

- **Bigonia P et al. (2006)** reported antiulcer activity to bark ethanol extract against experimental induced acute gastric ulcer model. The extract significantly reduced the ulcer index induced by both pyloric ligation and ethanol at 100 mg/ kg dose; similarly, extract treatment significantly reduced the secretary parameters such as volume and pH of gastric juice, free and total acidity. The extract has a significant effect on gastric mucous substances. By increasing total carbohydrate content and decreasing total protein content in pyloric ligated ulceration, all these effects are comparable to ranitidine (20 mg/kg). The results indicated that W. tinctoria bark extract protects gastric mucous membrane by improving microcirculation, increased capillary resistance or by precipitating microproteins.

- **Madhavan V et al. (2006)** reported that the aqueous and alcoholic extracts of the stem bark of W. tinctoria were investigated for their antipyretic and wound healing potential in albino rats using excision model. Wound healing activity was assessed by the percentage of wound contraction and the period of epithelization; there was wound contraction activity by using extracts from the 14^{th} day of topical application. Further, yeast induced pyrexia model was used for evaluation of antipyretic activity in albino rats and fall in body temperature of febrile rats was taken as an indication of antipyretic action. The drug extracts had significant antipyretic activity.

- **Sudhakar et al. (2006)** have tested the ethanol extracts of the leaves and flowers of *Cleome viscosa* for antimicrobial activity. Both extracts exhibited a broad spectrum of antimicrobial

activity, particularly significant against *Escherichia coli*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*, while the leaf extract showed moderate activity against pathogenic fungi.

- **Behera SK, Misra MK (2005)** reported that *B. malabaricum* prescribed to increase sperm count and to act as aphrodisiac.

- **Alluri V and Gottumukkala V. (2005)** reported that aqueous extracts of the plant *B. malabaricum* were screened for their cytotoxicity using the brine shrimp lethality test. The research supports that brine shrimp bioassay is trustworthy and convenient method for assessment of bioactivity of medicinal plants.

- **Alli PM et al. (2005)** reported that the fatty acid synthesis (FAS) had been found to be over express and hyperactive in most cancers.

4. METHODOLOGY:

1. *Bombax malabaricum* DC, *Cleome viscosa* Linn. will be collected from Kulish Smriti Van, Jaipur and *Wrightia tinctoria* Roxb. will be collected from University of Rajasthan campus, Jaipur and Identification of plants will be carried out with the help of NIRMAL INSTITUTE OF MEDICINAL AND AROMATIC PLANT CENTRE, Indore, M.P., INDIA.
2. *Bombax malabaricum* DC bark, *Cleome viscosa* Linn. and *Wrightia tinctoria* Roxb bark will be dried. The fine cured powder will be used as herbal drug. Weighed amount of dried powdered *Bombax malabaricum* DC bark, *Cleome viscosa* Linn. and *Wrightia tinctoria* Roxb bark will be extracted in different solvents by extraction methods.
3. Preliminary phytochemical investigation will be carried out using well established methods.
4. **Screening of extracted metabolites like anticancer and antioxidant activity will be done by following methods.**

4.1 *In vitro* antioxidant activities by following method

4.1.1 DPPH radical scavenging assay by Sreejayan & Rao, 1996

4.1.2 ABTS radical scavenging assay by Vaijanathappa, 2008

4.1.3 o-Phenanthroline assay by Sreejayan & Rao, 1996

4.1.4 Total antioxidant capacity by Shirwaikar *et al.*, 2004, Prieto et al., 1999

4.2 *In vitro* anticancer activity by following method

4.2.1 MTT cytotoxicity assay as per T.Mossman, 1983

4.2.2 Sulphorhodamine assay as per Eisenbrand *et al*, 2002

4.2.3 Trypan blue dye exclusion assay as per shapiro, 1998

4.2.3.1 Acridine orange/ethidium bromide staining as per Patsos *et al*. 2008

4.2.3.2 DNA fragmentation assay as per Singh *et al*, 1998

4.3 The safety of the extract(s)/fractions of plants by acute toxicity studies in mice/rats.

4.4 Selection of Animals for study: The Wistar albino rats of either sex weighing between 100-150 gm would be procured to study acute toxicity and anticancer activity studies. The animals would be stabilized for 1 week; in standard condition at room temp; 60±5% relative humidity and 12 light dark cycles. The Animal house facilities are approved by Institutional Animal Ethics Committee.

4.5 Acute toxicity study: The acute toxicity studies is conduct to determine the safe dose according to OECD Test Guidelines 425.

4.6 Selection of doses: The doses will be selected for the extracts is about 1/10th, 1/20th, 1/30th and 1/40th of the maximum tolerated safe dose found from acute toxicity studies.

4.7 *In vivo* anticancer activity by following method

4.6.1 EAC liquid tumor model

4.8 *Ex vivo* antioxidant activity

4.8.1 Reduced glutathione as per Ohkawa H *et al.*, 1979

4.8.2 Extent of lipid peroxidation as per Ohkawa H *et al.*, 1979

4.8.3 Glutathione-S- Transferase as per Townsend *et al*, 2003

4.8.4 Total thiols as per Miao, 1994

4.8.5 Catalase as per Aebi. 1983

5. Data will be tabulated and analyzed statistically

5. REFERENCES

1. Aebi H, Catalase IN, Burgmeyer HU. Methods of enzymatic analysis, 3rd Ed. New York: Academic Press; 1983 3: 273.
2. Ahmed salman el al. Analgesic and antiemetic activity of Cleome viscosa. Pak. J. Bot. 2011;43:119-122.
3. Alberts B. J. A. Cancer as a microevolutionary process, new york, garland science, 2002.
4. Aleykutty NA, Bindu AR, Sangeetha S, Jiljit G. Evaluation of anti-inflammatory and analgesic activity of Wrightia tinctoria leaves. J Biol Act Prod Nat 2011; 1: 33-41.
5. Alli PM, Pinn ML, Jaffee EM, Mcfadden JM and Kuhaida FP. Fatty acid synthase inhibitors are chemopreventive for mammary cancer in neu-N transgenic mice. Oncogene 2005; 24(1):39–46.
6. Alluri V and Gottumukkala V. Assessment of Bioactivity of Indian Medicinal Plants using Brine Shrimp (Artemia salina) Lethality Assay. IJASE 2005; 3(2):125-134.
7. Ames, B. N. 1989. Endogenous oxidative dna damage, aging, and cancer. Free radic res commun, 1989;7, 121-8.
8. Anburaj jeyaraj et al. In vitro regeneration of Cleome viscosa – an important medicinal herb. Journal of cell and molecular biology, 2011;9:37-44.
9. Aparadh V.T., Naik V.V., Karadge B.A. Antioxidative properties within some Cleome species. Annali D botanica. 2012;2:49-56.
10. Balachandran, P. & Govindarajan, R. Cancer an ayurvedic Perspective. Pharmacol res,2005; 51, 19-30.
11. Bawa AS, Khanum F, Rakesh KS, Rajesh A. Herbal drugs: a twenty first century perspective. New Delhi: JP Brothers Medical Publishers; 2006.
12. Bawankule DU, Chattopadhyay SK, Pal A, Saxena K, Yadav S, Yadav NP, Srivastava A, Gupta AK, Khanuja SPS. An in-vivo study of the immunomodulatory activity of coumarinolignoids from Cleome viscosa. Nat Prod Commun,2007 2: 923–926.
13. Behera SK, Misra MK. Indigenous phytotherapy for genito-urinary diseases used by the Kandha tribe of Orissa, India. Journal of Ethnopharmacology 2005; 102:319–325.
14. Berwick, M. & Vineis, P. 2000. Markers of dna repair and susceptibility to cancer in humans: an epidemiologic review. J natl cancer inst,2000;92, 874-97.

15. Bhuvaneshwar Upadhyay, Parveen, Anil K. Dhaker and Ashwani Kumar. Ethnomedicinal and ethnopharmaco-statistical studies of Eastern Rajasthan, India. Journal of Ethnopharmacology 2010; 129:64–86.
16. Bigoniya P, Rana AC, Agrawal GP. Evaluation of the antiulcer activity of hydro-alcoholic extract of Wrightia tinctoria bark in experimentally induced acute gastric ulcers on rat. Niger J Nat Prod Med 2006; 10: 36-40.
17. Bigoniya P, Rana AC, Lariya S. Immunomodulatory activity of Wrightia tinctoria bark alcoholic extract on rats. Curr Pharm Res J 2007; 1: 1-9.
18. Bigoniya P, Rana AC. Antidiarrheal and antispasmodic activity of Wrightia tinctoria bark and its steroidal alkaloid fraction. Pharmacologyonline 2009; 3: 298-310.
19. Bigoniya P, Shukla A, Agrawal GP, Rana AC. Pharmacological screening of Wrightia tinctoria bark hydro-alcoholic extract. Asian J Exp Sci 2008; 22: 235-244.
20. Bigoniya P. Rana AC. Protective effect of Wrightia tinctoria bark triterpenoidal fraction on carbon tetrachloride-induced acute rat liver toxicity. Iran J Pharmacol Ther 2010; 9: 55-62.
21. Boddapati Srinivasa Rao et al. Effects of Cleome viscosa on hyperalgesia, oxidative stress and lipid profile in STZ induced diabetic neuropathy in Wistar rats. Pak. J. Pharm. Sci. 2014;27(5):1137-1145.
22. Brown R, Wrightia, Flora of China., 1995; 16: 174–175.
23. Brummit RK. Vascular plants families and genera. Kew: Royal Botanic Gardens; 1992.
24. Chen Jun, Zhuang Donghong, Cai Weijia and Sugiyama Kazuo et al. Inhibitory Effects of Four Plants Flavonoids Extracts on Fatty Acid Syntheses. JES Supplement 2009; 131–134.
25. Compendium of Indian medicinal plants: volume I, pp. 61.
26. Dama GY, Tare HL, Gore MS, Deore SR, Bidkar JS. Comparative helmintholytic potential of extracts obtained from Cymbopogon citratus and Wrightia tinctoria leaves. Int J Pharm Bio Sci 2011; 2: 321-327.
27. Dang R, Sabitha JS, Shivananda BG. Screening antibacterial activity of Calendula officinalis, Wrightia tinctoria, Cassia tora and Azadirachta indica on Staphylococcus aureus and Escherichia coli. Biomed 2006; 1: 56-58.
28. Divakar MC, Lakshmi SD. Antiulcer activity of Wrightia tinctoria (Roxb.) R. Br. Der Pharmacia Sinica 2011; 2: 355-360.
29. Dormandy, T. L. 1978. Free-radical oxidation and antioxidants. Lancet,1978;1, 647-50.

30. Edwards, B. K., Ward, E., Kohler, B. A., Eheman, C., Zauber, A. G., Anderson, R. N., Jemal, A., Schymura, M. J., Lansdorp-Vogelaar, I., Seeff, L. C., Van Ballegooijen, M., Goede, S. L. & Ries, L. A. Annual Report to the nation on the status of cancer, 1975-2006, featuring colorectal cancer trends and impact of interventions (risk factors, screening, and treatment) to reduce future rates. Cancer,2010;116, 544-73.
31. Evert J.. Overview: introduction to cancer. Available from: www.mentalhelp.net. 2016
32. Faizan mohammad and Mazumder avijit. Wound healing potentiality of methanolic extract of arieal part of Cleome viscosa. International journal of pharm tech research. 2013;5(3):978-982.
33. Florence, B., Handrow, R. & Laughon, A. DNA-binding specificity of the fushi tarazu homeodomain. Mol cell biol,1991;11, 3613-23.
34. Frederich M, Tits M, Angenot L. Potential antimalarial activity of indole alkaloids. Trans R Soc Trop Med Hyg 2008; 102: 11-19.
35. Gautam, M., Saha, S., Bani, S., Kaul, A., Mishra, S., Patil, D., Satti, N. K., Suri, K. A., Gairola, S., Suresh, K., Jadhav, S., Qazi, G. N. & Patwardhan, B. Immunomodulatory activity of asparagus racemosus on systemic th1/th2 immunity: implications for immunoadjuvant potential. J ethnopharmacol,2009;121, 241-7.
36. Gautam, R., Saklani, A. & Jachak, S. M. Indian medicinal plants as a source of antimycobacterial agents. J ethnopharmacol,2007;110, 200-34.
37. Ghimire K, Bastakoti RR. Ethnomedicinal knowledge and healthcare practices among the Tharus of Nawalparasi district in central Nepal. FEM 2009; 257:2066–2072.
38. Gill BS, Singhal VK, Bedi YS and Bir SS. Cytological evolution in the woody taxa of Pachmarhi Hills. J Cytol Genet., 1990; 25: 308–320.
39. Hanahan D. & weinberg R. A. The hallmarks of cancer. Cell, 2000; 100, 57-70.
40. Hopkins, K. Understanding cancer biodiversity. Howard hughes medical institute, 2010.
41. Hossain E, Mandal SC and Gupta JK. Phytochemical Screening and In-vivo Antipyretic Activity of the Methanol Leaf-Extract of Bombax Malabaricum DC (Bombacaceae). TJPR 2011; 10(1):55-60.
42. Huang, S. Y., Yao, M., Tang, J. L., Lee, W. C., Tsay, W., Cheng, A. L., Wang, C. H., Chen, Y. C., Shen, M. C. & Tien, H. F. Epidemiology of Multiple myeloma in taiwan: increasing incidence for the past 25 years and higher Prevalence of extramedullary myeloma in patients younger than 55 years. Cancer,2007;110, 896-905.

43. Jain PS, Bari SB. Anti-inflammatory effects of wood stem extracts of Wrightia tinctoria. Asian J Tradit Med 2010; 5: 132-137.
44. Jain PS, Bari SB. Evaluation of wound healing effect of petroleum ether and methanolic extract of Abelmoschus manihot (L.) Medik. Malvaceae and Wrightia tinctoria R. Br., Apocynaceae in rats. Braz J Pharmacogn 2010; 20: 756-761.
45. Jeyachandran R, Baskaran X, Cindrella L. Screening of phytochemical and antibacterial potential of four Indian medicinal plants. Libyan Agric Res Center J Int 2010; 1: 301-306.
46. Kannan P, Shanmugavadivu B, Petchiammal C, Hopper W. In vitro antimicrobial activity of Wrightia tinctoria leaf extracts against skin microorganisms. Acta Bot Hung 2006; 48: 323-329.
47. Keshri G, Kumar S, Kulshreshtha DK, Rajendran SM, Singh MM. Postcoital interceptive activity of Wrightia tinctoria in Sprague- Dawley rats: a preliminary study. Contraception 2008; 78: 266-270.
48. Khadiza khanam et al. Studies on antioxidant, analgesic, anti-inflammatory and CNS depressant activities of the plant Cleome viscosa linn. International journal of innovative pharmaceutical sciences and research. 2015;3:12-28.
49. Khan K, Sharma SK, Ramarao S and Purohit SD, Karyomorphological Studies in Wrightia tomentosa and W. tinctoria, Multipurpose Tree Species of Aravallis in South-East Rajasthan. Cytologia, 2011.; 76(2): 125-128.
50. Khyade MS, Vaikos NP. Antibacterial evaluation and phytochemical analysis of Wrightia tinctoria (Roxb.) R. Br. leaves. Pharmacologyonline 2009; 2: 808-813.
51. Krishnamoorthy JR, Ranganathan S, Shankar SG, Ranjith MS. Dano: a herbal solution for dandruff. Afr J Biotechnol 2006; 5: 960-962.
52. Kumar DL, Rao KN, Bindu M, Kumar SD, Banji D. Anti oxidation activity of Wrightia tinctoria Roxb. bark and Schrebera swietenoides Roxb. bark extract. J Pharm Res 2011; 4: 396-397.
53. Kumar DL, Rao KN, Madhavi B, Kumar DS, Banji D. Alphaglucosidase inhibitory activities of Wrightia tinctoria Roxb and Schrebera swietenoides Roxb. bark extract. Res J Pharmacol Pharmacodyn 2011; 3: 22-24.
54. Kviecinski, M. R., Felipe, K. B., Schoenfelder, T., De Lemos Wiese, L. P., Rossi, M. H., Goncalez, E., Felicio, J. D., Filho, D. W. & Pedrosa, R. C. Study of the antitumor potential of bidens pilosa (asteraceae) used in brazilian folk medicine. J ethnopharmacol,2008;117, 69-75.
55. Laura dungo, Y. N., Angelo Azzi. Antioxidants, john willey & sons. 2011

56. M. A. H. Mollik, M. F. Hossain, D. Sen, A. I. Hassan and M. S. Rahman. Traditional Asian medicine & leprosy in Bangladesh. European JIM 2009; 1:181–221.
57. Madhavan V, Tomar AS, Murali A, Yoganarasimhan SN. Wound healing and antipyretic activity of stem bark of Wrightia tinctoria. J Trop Med Plants 2006; 7: 69-73.
58. Mali RG, Mahajan SG, Mehta AA. In vitro screening of Cleome viscosa extract for anthelmintic activity. Pharm Biol,2007 45: 766–768.
59. Nadkarni. The indian materia medica, bombay, popular prakashan. 1982
60. Newman D. J., Cragg G. M. & Snader, K. M. Natural products as sources of new drugs over the period 1981-2002. J nat prod,2003; 66, 1022-37.
61. Ngan PT, A revision of the genus Wrightia (Apocynaceae). Ann Miss Bot Gard., 1965, 52: 114–175.
62. Nima D. Namsa, Hui Tag, M. Mandal, P. Kalita and A.K.Das. An ethnobotanical study of traditional anti-inflammatory plants used by the Lohit community of Arunachal Pradesh, India. Journal of Ethnopharmacology 2009; 125:234–245.
63. Ohkawa H, Onishi N, Yagi K. Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. Anal Biochem. 1979, 95:351–358.
64. Orwa C, Mutua A, Kindt R, Jamnadass R and Anthony S, Agroforestree Database: a tree reference and selection guide version 4.0 (World Agroforestry Centre, Kenya), 2009.
65. Panno, J. Cancer: the role of genes, lifestyle, and environment, new york, facts on file, inc. 2005
66. Park, H. J., Kim, M. J., Ha, E. & Chung, J. H. Apoptotic effect of hesperidin through caspase3 activation in human colon cancer cells, snu-c4. Phytomedicine,2008;15, 147-51.
67. Ponnusamy K, Ramasamy M, Savarimuthu I, Paulraj MG. Indirubin potentiates ciprofloxacin activity in the NorA efflux pump of Staphylococcus aureus. Scand J Infect Dis 2010; 42: 500-505.
68. Raj RA, Kumar AS, Gandhimathi R. Anti-diabetic effect of Wrightia tinctoria extracts in streptozotocin-induced diabetic rats. Int J Phytopharmacol 2010; 1: 47-52.
69. Raj RA, Kumar AS, Gandhimathi R. Hypoglycemic and hypolipidemic activity of Wrightia tinctoria L. in alloxan induced diabetes in albino wistar rats. Pharmacologyonline 2009; 3: 550-559.

70. Raju AJS, Zafar R and Rao SP. Floral device for obligate selfing by remote insect activity and anemochory in Wrightia tinctoria (Roxb.) R.Br. (Apocynaceae), Curr Sci., 2005, 88(9), 1378-1380.
71. Raman R Chandak et al. In vitro evaluation of anthelmintic potential of leaves of Cleome viscosa linn. International journal of pharmaceutical sciences review and references. 2010 November – December; 5:77-79.
72. Rao PS, Maheswar Rao G and Satyanarayana VVV. Rooting of stem cuttings of Wrightia tinctoria (Roxb.) R.Br.: An important medicinal plant. Indian Forester., 1999; 125(4): 427-428.
73. Ravi V, Patel SS, Varma NK, Dutta D. and Saleem TSM. Hepatoprotective Activity of Bombax ceiba Linn against Isoniazid and Rifampicin-induced Toxicity in Experimental Rats. IJARNP 2010; 3(3):19-26.
74. Reddi CS, Reddi EUB and Reddi MS. A novel mechanism of pollination in Wrightia tinctoria R.Br. Curr Sci., 1979; 48(16): 746-747.
75. Reed, J. C. & Pellecchia, M. Apoptosis-based therapies for hematologic malignancies. Blood,2005;106, 408-18.
76. Ruddon R. W., Cancer biology, oxford university press 2007.
77. S.Vimal Kumar, A.J.M.Christina, P.V.GeethaRani, G. Nalini, N.Chidambaranathan. Antifibrotic effect of Cleome viscosa Linn on Carbon tetra chloride (CCl4) induced liver fibrosis. Der Pharma Chemica. 2009;1(2): 92-96.
78. Sangeetha S et al. Comparative evaluation of free radical scavenging activity of Cleome viscosa and trichodesmaindicum. International journal of pharmacy and pharmaceutical sciences. 2014;6:318-325.
79. Sarada S, Nair GS and Reghunath BR. Quantification of medicinally valuable weeds in oil palm plantations of Kerala. Journal of Tropical Agriculture., 2002; 40: 19-26.
80. Sathianarayanan S, Jose A, Rajasekaran A, Rijo M, George A, Chittethu B. Diuretic activity of aqueous and alcoholic extracts of Wrightia tinctoria. Int J Phytopharmacol 2011; 2: 7-8.
81. Selvam P, Murugesh N, Witvrouw M, Keyaerts E, Neyts J. Studies of antiviral activity and cytotoxicity of Wrightia tinctoria and Morinda citrifolia. Indian J Pharm Sci 2009; 71: 670-672.
82. Sengottuvelu S, Duraisamy R, Nandhakumar J, Sivakumar T. Hepatoprotective activity of Cleome viscosa against carbon tetrachloride induced hepatotoxicity in rats. Pharmacog Mag,2007 3: 121–124.

83. Shankara SR, Rangarajana R, Sarada DV, Sreenath KC. Evaluation of antibacterial activity and phytochemical screening of Wrightia tinctoria L. Pharmacogn J 2010; 2: 19-22.
84. Shapiro 1988. Practical flow cytometry, New York, John Wiley & Sons.
85. Shruthi A, Latha KP, Vagdevi HM, Vaidya VP, Pushpa B. In vitro anthelmintic activity of leaves extract of Wrightia tinctoria. Int J ChemTech Res 2010; 2: 2043-2045.
86. Singh PD, West ME. Pharmacological investigations of sticky viscome extract (Cleome viscosa Linn) in rats, mice and guineapigs. Phytother Res,1991 5: 82–84.
87. Sreejayan N, Rao MNA. Free radical scavenging activity of curcuminoids. Drug Res . 1996; 46: 169.
88. Sudhakar M, Rao CV, Rao PM, Raju DB. Evaluation of antimicrobial activity of Cleome viscosa and Gmelina asiatica. Fitoterapia,2006 77: 47–49.
89. Suresh DR, Hegde GV and Ganesh KB, Vegetative propagation of Wrightia tinctoria R.Br. - An important multipurpose tree. Indian Forester., 2004; 130(8): 950-952.
90. Thabah P, Murugananthan G, Joshi NC, Nandakumar K, Lakshman K, Talwar S. Immunomodulatory activities of Wrightia tinctoria (Roxb.) R. Br. bark extracts. Pharmacologyonline 2009; 3: 663-669.
91. Tharkar PR, Tatiya AU, Surana SJ, Bhajipale NS. Antiinflammatory studies of Wrightia tinctoria R. Br. stem bark in experimental animal models. Int J PharmTech Res 2010; 2: 2434-2437.
92. The Ayurvedic Pharmacopoeia of India, Part I, Government of the health and family welfare, New Delhi, vol. 3, pp. 183-184.
93. Toh C. K. & Lim W. T. Lung cancer in never-smokers. J clin pathol,2007; 60, 337-40.
94. Upadhyay aadesh et al. Topical application of Cleome viscosa increases the expression of basic fobroblast growth factor and type III collagen in rat cutaneous wound. Hindawi Publishing, Corporation BioMed Research International Volume 2014, Article ID 680879, 7.
95. Upadhyay R.K. Antimicrobial effect of Cleome viscosa and Trigonella foenum graecum seed extract. Journal of cell and tissue research. 8:1355-1360.
96. V. Jain, S.K. Verma, S. S. Katewa, S. Anandjiwala and B. Singh. Free radical scavenging property of Bombax ceiba Linn. Root. Research journal of medicinal Plants2011; 5 (4): 462-470.
97. Vaghasiya Y and Chanda S. Screening of same traditionally used Indian plants for antibacterial activity against Klebsiella Pneumoniae. JHMT 2009; 3(2):161-164.

98. Valiante N. Use of tryptanthrin compounds for immunepotentation; 2004. [Online] [Accessed on 10 January, 2016]
99. Verpoorte R. Pharmacognosy in the new millennium: leadfinding and biotechnology. J pharm pharmacol,200;52, 253-62.
100. Vijaykumar R, Muthukumar C, Kumar T, Saravanamuthu R. Characterization of Malassezia furfur and its control by using plant extracts. Indian J Dermatol 2006; 512: 145-148.
101. Wall. Campothesin, academic press inc. 1980
102. YW H. Induction of apoptosis in human hepatocarcinoma smmc-7721 cells in vitro by flavonoids from astragalus complanatus. J. ethnopharmacol.,2009;123, 293-301.
103. Zito SW, Kunaparaju N, Taldone T, Shinde J. Inhibition of ocular aldose reductase by extracts of ayurvedic herbs. Invest Ophthalmol Vis Sci 2007; 48: 5833.